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Assessment of Kidney Function Indices in Male Albino Wistar Rats Administered Ethanol Stem Extract of *Dennettia tripetala* (Pepper fruit)

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Abstract

This study was carried out to assess some kidney function indices in Wistar rats administered ethanol stem extract of *Denettia tripetala*. The parameters assessed were creatinine, urea, and electrolytes (sodium, potassium, chloride, bicarbonate). Thirty-two albino Wistar rats divided into 4 groups of eight rats each were used in this study. Group 1 was the control group, group II received an oral dose of 100 mg/kg, ethanol stem extract of *Denettia tripetala* daily, group III received an oral dose of 200 mg/kg ethanol stem extract of *Dennettia tripetala* daily while group IV received an oral dose of 300 mg/kg ethanol stem extract of *Dennettia tripetala* daily. After 21 days of treatment the rats were sacrificed and standard analytical methods were used to assay the biochemical parameters. The result of creatinine (mol/L) showed a significant ($P<0.05$) increase in the treatment groups (9.18 ± 1.22, 12.42 ± 0.87, 14.56 ± 1.46) compared to the control (4.21± 0.65) while urea (mol/L) showed significant ($P<0.05$) decrease in group III and IV (2.76± 1.12, 1.61 ± 0.92) compared to the control (6.34 ± 0.25). The electrolytes (Na^+ and Cl^-) showed significant ($P<0.05$) increase in the treatment groups compared to the control while K^+ (mmol/L) was not significant ($p>0.05$) compared to the control. However, bicarbonate (mmol/L) showed a significant ($P<0.05$) decrease in group II and group III compared to the control. The result of this study has shown that administration of ethanol stem extract of *Dennettia tripetala* may induce toxicity to the kidney which may hamper renal function.

Keywords: *Dennettia tripetala;* Electrolytes; Toxicity; Renal function; Wistar rats

Introduction

Dennettia tripetala is a plant widely grown in the rain forest zones of Nigeria, some part of West Africa and sometimes in Savannah [1,2]. The plant popularly called pepper fruit tree is known in Southern Nigeria local languages as Nkarika (Ibibio/Efik), Ako (Edo), Nmimi (Ibo) and Ata Igbere (Yoruba) [3]. *Dennettia tripetala* is an indigenous fruit tree which belongs to the family Annonaceae [4]. The tree can grow to the height of 12-15 m and have a girth of 0.6 m with fruit which are green when developing but turn red with ripening; also the leaves are 3-6 inches long and 1.5-2.5 inches broad and elliptic in shape [1,5,6]. *Dennettia tripetala* has been used for both medicinal and cultural purpose. Reports indicated that it has been used for the treatment of various ailments [5,6] and the entertain guest during marriage ceremonies [7-9].

The use of *Dennettia tripetala* fruit, seed and stem for medicinal purposes has been attributed to the presence of phytochemicals, like tannins, alkaloid, steroid, flavonoids, cardiac glycosides, saponins and terpenoids [10,11]. The presence of these phytochemicals have been widely reported to possess antibiotic [12,13], antioxidant [1,14,15], anti-inflamatory [3,16] potentials. The hypoglyceaemic, analgesic, hypolipidaemic and haematoxic effects of *Dennettia tripetala* fruits have been reported [17-19].

The kidney is a very important organ of the body which helps in the maintenance of the body's homeostasis. It plays a principal role in excretion of waste products of the body's metabolism, drugs and chemicals [20]. Toxic substances such as chemicals, drugs, heavy metals, and immunological complexes can inflict injury on the kidney and in turn incapacitate it from performing notable excretory functions that may lead to renal failure [21]. Creatinine and urea are non-protein nitrogenous metabolites that are cleared from the body by the kidney following glomerular filtration. Estimates of plasma or serum levels of these metabolites and electrolytes are usually employed as a marker for kidney function [22].

The medicinal value of different parts of *Dennettia tripetala* tree (fruit, seed, stem and root), has gained wide acceptability in communities in South-Eastern Nigeria where alternative medicine is prevalent because of high level of poverty, poor education and lack of access to quality health care [23]. The stem is used as an antimicrobial agent to treat tissue inflammation, fever, hyperglycaemia and its attendant health challenges. In view of the above, this study was designed to assess the kidney function indices in male albino wistar rats administered ethanol stem extract of *Dennettia tripetala* so as to document possible effects or otherwise on renal function.

Material and Methods

Plant materials

Fresh stem of *Dennettia tripetala* were obtained from different local farmlands in Umuonina, Umudagu Mbieri in Mbaitoli L.G.A of Imo State Nigeria. They were identified and authenticated in the department of Plant Science and Biotechnology, Imo State University, Owerri. The stem was chopped to pieces with knife (for effective drying) and washed under running tap water to remove un-wanted debris. It was later dried under shade at room temperature for 5 weeks until constant

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weight was obtained. The dried stem was ground to fine powder with a mechanical grinder and kept in labelled airtight containers under dry conditions until required for use.

Ethanol stem extraction of *Dennettia tripetala*

The ethanol stem extraction of *Dennettia tripetala* was done using the modified method of Abdulrahman et al. [24]. Two hundred grams of grounded stem of *Dennettia tripetala* was macerated in 1 litre of absolute ethanol for 72 hours. It was filtered with sterile filter paper (Watman No. 1) after which it was evapourated to dryness at 40°C in a vaccum using a rotary evapourator. Approximate concentrations of the extract were constituted to the required doses for the treatment of the animal using normal saline.

Animals

Male albino rats of wistar strain weighing between 150 g-190 g were used for the study. The rats purchased from Faculty of Agriculture, Imo State University, were kept in the animal house of department of Biochemistry, Imo State University, Owerri. The rats were acclimatized to daily handling for seven (7) days and were fed ad-libitum with normal rat chow (Product of Vital Feed Nigeria Ltd) and water.

Experimental design

Thirty-two rats were used in the study. They were randomly assigned into four groups of eight rats per group.

Group 1: The rats in this group were orogastrically given 1 ml of normal saline daily for 21 days in addition to having free access to normal rat chow and water ad-libitum. They serve as the control.

Group 11: The rats in this group were orogastrically given 1 ml equivalent to 100 mg/kg body weight of *Dennettia tripetala* stem extract daily for 21 days. They also had free access to normal rat chow and water ad-libitum.

Group III: The rats in this group were orogastrically given 1 ml equivalent to 200 mg/kg body weight of *Dennettia tripetala* stem extract daily for 21 days. They also had free access to normal rat chow and water ad-libitum.

Group IV: The rats in this group were orogastrically given 1 ml equivalent to 300 mg/kg body weight of *Dennettia tripetala* stem extract daily for 21 days. They also had free access to normal rat chow and water ad-libitum.

Analytical procedure

Twenty-four hours after the end of the last treatment the rats were anaesthetized with chloroform and with sharp scissors, their cavities were cut open to expose the heart. By cardiac puncture of each rat, blood sample was collected with a sterile syringe into a plain sterile test tube and allowed to clot for 10 minutes. The serum was separated by spinning at 1000 rpm for 5 minutes with Wisperfuge model 1384 centrifuge (Samson, Holland) and collected with a Pasteur pipette into clean labeled sample bottle. The serum was used for biochemical analysis.

Biochemical analysis

Kidney function indices were analyzed from the sample. Urea concentration was determined by the diacetyl monoxime method using assay kit from Randox laboratories UK while creatinine concentration was determined by the alkaline picrate method [25]. Determination of serum sodium and potassium concentration were done using reagent kit [25] serum bicarbonate concentration was determined trtrimetrically while mercuric nitrate method was used to determine the concentration of chloride [26] as modified by Teco diagnostics 1268N Lakeview Avenue Anahein; CA 92807, USA.

Statistical analysis

Data generated from the study were presented as mean ± SD of four determinations. Statistical analysis was done by one way analysis of variance using the SPSS version 21.0. This was followed by student's t-test of significance. The mean difference at $P<0.05$ were considered statistically significant.

Results

The result on Table 1 showed the effect of ethanol stem extract of *Dennettia tripetala* on serum urea and creatinine. There were significant ($P<0.05$) decrease in serum urea on rats treated with 200 mg/kg and 300 mg/kg stem extract while the rats treated with 100 mg/kg showed insignificant ($P>0.05$) decrease compared to the control. Conversely, serum creatinine showed significant ($P<0.05$) increase in all the treatment groups compared to the control. The effects of ethanol stem extract of *Dennettia tripetala* on serum electrolytes of albino wistar rats are shown in Table 2. The result showed a significant ($P<0.05$) increase on serum sodium and chloride concentrations in test group compared to the control while serum bicarbonate concentration showed a significant ($P<0.05$) decrease in rats treated with 200 mg/kg and 300 mg/kg of stem extract of *Dennettia tripetala* compared to control. Serum potassium showed insignificant ($P>0.05$) decrease in treatment groups compared to the control.

Discussion

The kidney as an excretory organ is central to the normal functioning of the body. Its role in maintenance of the body homeostasis, excretion of waste products of metabolism, drugs and chemical are vital to maintenance of health [20]. Among the waste products of metabolism excreted by the kidney are urea and creatinine while in the tubules electrolytes are reabsorbed in maintenance of body's homeostasis. Creatinine and urea are non-protein nitrogenous metabolites that are cleared by the body following glomerular filtration, thus assessment of serum urea, creatinine and electrolytes ($Na+$, K^+, Cl^-, HCO_3^-) are vital and sensitive biochemical markers which are usually employed in the diagnosis of renal failure and damage [21,22,27].

The major non-protein nitrogenous catabolite of protein

Group	Treatment	Urea (mol/L)	Creatinine (mol/L)
I	1 ml of normal saline	6.34 ± 0.25	4.21 ± 0.65
II	1 ml of 100 mg/kg stem extract of *Dennettia tripetala*	5.18 ± 0.39	9.18 ± 1.22*
III	1 ml of 200 mg/kg stem extract of *Dennettia tripetala*	2.76 ± 1.12*	12.42 ± 0.87*
IV	1 ml of 300 mg/kg stem extract of *Dennettia tripetala*	1.61 ± 0.92*	14.56 ± 1.46*
Values are mean of four determination ± SD. n=8; *Significantly different from control ($P<0.05$)			

Table 1: Effect of ethanol stem extract of *Dennettia tripetala* on serum Urea and Creatinine.

Group	Treatment	Sodium (mmol/L)	Potassium (mmol/L)	Cl^- (mmol/L)	HCO_3^- (mmol/L)
I	1 ml of normal saline	143.20 ± 2.65	4.16 ± 0.80	96.24 ± 0.70	24.81 ± 1.62
II	1 ml of 100 mg/kg stem extract of *Dennettia tripetala*	157.46 ± 1.82*	4.54 ± 0.52	112.67 ± 1.84*	22.26 ± 0.87
III	1 ml of 200 mg/kg stem extract of *Dennettia tripetala*	168.14 ± 2.41*	3.86 ± 0.14	121.26 ± 2.21*	16.49 ± 1.32*
IV	1 ml of 300 mg/kg stem extract of *Dennettia tripetala*	179.64 ± 2.88*	3.79 ± 0.16	130.31 ± 3.22*	14.07 ± 2.26*
Values are mean of four determinations ± SD. n=8; *Significantly different from control.(P<0.05)					

Table 2: Effect of ethanol stem extract of *Dennettia tripetala* on serum electrolytes.

metabolism is urea. In this study, the administration of 200 and 300 mg/kg ethanol stem extract of *Dennettia tripetala* showed a significant (P<0.05) decrease in urea compared to control. This suggests either that the urea cycle may have been affected by plant extract leading to the reduction in the production of urea [10,27] or alkaloids and saponins contained in the plant extract [1,10] has produced systemic toxicity, invariably leading to reduced ability to excrete waste, and failure to maintain balance in body fluid and electrolytes [28]. Similar results were reported [29], on *Alstonia boonei* stem bark on Guinea pig and [30] on root and leave extracts of *Sida acuta* on wistar rats. Creatinine clearance in the glomerulus of the kidney is a useful tool to assessing the functionality of the kidney [31,32]. Creatinine is produced endogenously in the muscle by a non-enzymic action on creatine phosphate. The result of creatinine obtained in this study showed a significant (P<0.05) increase on administration of ethanol stem extract of *Dennettia tripetala* compared to the control. The significant increase in creatinine may have resulted from glomerular inflammation and interstitial nephritis, though the exact mechanism was not covered in this study. This result corroborate the result of [33] on extract of *Acalypha wilkesiana* and [34] on extract of *Alstonia boonei* on wistar rats. Their reports suggested that the presence of tissue damaging alkaloid and saponins may have contributed to the increase in creatinine concentration.

Body fluid compartments (both extracellular and intracellular fluids) comprises of inorganic electrolytes which in its dissociated forms help to facilitate the movement of water and electrolytes between the body fluid compartments [35]. In this study, administration of ethanol stem extract of *Dennettia tripetala* significantly increased (P<0.05) sodium and chloride concentration while bicarbonate concentration was significantly decreased (P<0.05) when compared to the control. The hypernatramic effect may be due to the excessive loss of water from the body fluid. From the result of this study, the concentration of potassium was not significantly decreased (P>0.05) which may confer the possibility of the hypernatraemic effect to the Na^+/H^+ exchanger instead of Na^+/K^+ pump [30,36]. The membrane-bound aldosterone regulates the absorption of sodium into the cell while Na^+/K^+ pump may have been impaired on the administration of stem extract of *Dennettia tripetala.* This is also supported by the significant decrease (P<.0.05) in bicarbonate concentrations observed in this study, suggesting that the stem extract may have induced renal damage resulting to impairment of renal function.

Conclusion

From the result of this study, the significant changes in the kidney function indices showed that the stem extract of *Dennettia tripetala* may pose glomerular and tubular dysfunction of the nephron which indicates that the herbal preparation may contribute to renal failure and as such should not be taken without proper medical advice and monitoring.

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